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(71) Applicant: THE GOVERNMENT OF THE UNITED STATES OF AMERICA, represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; Box OTT, Bethesda, MD 20892 (US).

(72) Inventors: PASTAN, Ira; 11710 Beall Mountain Road, Potomac, MD 20854 (US). BENHAR, Itai; 341 Congressional Lane, Rockville, MD 20852 (US). PADLAN, Eduardo, A.; 4006 Simms Drive, Kensington, MD 20895 (US). JUNG, Sun-Hee; 259 Congressional Lane #206, Rockville, MD 20852 (US). LEE, Byungkook; 10711 Sandy Landing Road, Potomac, MD 20854 (US). WILLINGHAM, Mark; 108 Kimberwicke Drive, Summerville, SC 29483 (US). FITZGERALD, David; 1202 Azalea Drive, Rockville, MD 20850 (US). BRINKMANN, Ulrich; 4404 Everett Street, Kensington, MD 20895 (US). PAI, Lee; 34 Tivoli Lake Street, Silver Spring, MD 20906 (US).

(74) Agents: WEBER, Ellen, Lauver et al.; Townsend and Townsend and Crew, Steuart Street Tower, One Market, San Francisco, CA 94105-1492 (US).

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(54) Title: TUMOR-SPECIFIC ANTIBODY FRAGMENTS, FUSION PROTEINS, AND USES THEREOF

(57) Abstract

This invention provides for recombinant single chain antibodies capable of specifically binding to a Lewis^Y-related carbohydrate antigen and fusion proteins comprising these antibodies. More particularly, the invention provides for single chain Fv (ScFv) regions of the monoclonal antibodies B1, B3, and B5, humanized single chain Fv regions of B1, B3, and B5, and fusion proteins comprising these scFv regions. The invention also provides for a number of stabilizing mutations of the Lewis^Y-binding monoclonal antibody B3. In addition, the invention provides for methods of detecting cells bearing a Lewis^Y antigen in a patient and for methods of killing or inhibiting the growth of cells bearing a Lewis^Y antigen in a patient. The invention also provides for a method of improving the binding affinity of antibodies lacking a serine at position 95 of the V_H region that involves mutating position 95 to a serine.

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72. A single chain fusion protein comprising:

a) a humanized Fv region of both the light and heavy chains of an antibody selected from the group consisting of B1, B3 and B5; and

b) an effector molecule;

wherein the humanized Fv region and the effector molecule are recombinantly fused to form a single molecule.

73. The fusion protein of claim 72, wherein the Fv region of said protein comprises a humanized variable heavy chain having the amino acid sequence designated HumB3V_H in Figure 11.

74. The fusion protein of claim 72, wherein the Fv region of said protein comprises a humanized variable heavy chain having the amino acid sequence designated HumB3V_L in Figure 11.

75. The fusion protein of claim 72, wherein the Fv region of said protein comprises a humanized variable heavy chain having the amino acid sequence designated HumB3V_H in Figure 11 and a humanized variable light chain having the amino acid sequence designated HumB3V_L in Figure 11.

76. The fusion protein of claim 72, wherein the Fv region of said protein comprises a humanized variable heavy chain having the amino acid sequence designated HumB3V_H in Figure 11 with the exception that the sequence has an arginine at a position in said variable heavy chain designated as 82b in Figure 11 replaced serine with arginine at the position in the variable heavy chain designated as 82b in Figure 11.

77. The fusion protein of claim 72, wherein said effector molecule is a *Pseudomonas* exotoxin.

78. The fusion protein of claim 77, wherein said *Pseudomonas* exotoxin is PE38, PE40, PE38KDEL and PE38REDL.

79. A recombinant DNA molecule that encodes a humanized Fv region of both the light and heavy chains of an antibody wherein said antibody is a monoclonal antibody selected from the group consisting of B1, B3 and B5.

80. The recombinant DNA molecule of claim 79, wherein said DNA sequence encodes an Fv region comprising a humanized variable heavy chain having the amino acid sequence designated $HumB3V_H$ in Figure 11.

81. The recombinant DNA molecule of claim 79, wherein said DNA sequence encodes an Fv region comprising a humanized variable light chain having the amino acid sequence designated $HumB3V_L$ in Figure 11.

82. The recombinant DNA molecule of claim 79, wherein said DNA sequence encodes an Fv region comprising a humanized variable heavy chain having the amino acid sequence designated $HumB3V_H$ in Figure 11a and a humanized variable light chain having the amino acid sequence designated $HumB3V_L$ in Figure 11.

83. The recombinant DNA molecule of claim 79, wherein said DNA sequence encodes an Fv region comprising a humanized variable heavy chain having the amino acid sequence designated $HumB3V_H$ in Figure 11A with the exception that there is an arginine at a position in said variable heavy chain designated as 82b in Figure 11.

84. A recombinantly produced protein comprising a humanized Fv region of both a light and a heavy chain of an antibody wherein said antibody is a monoclonal antibody selected from the group consisting of B1, B3 and B5.

85. The protein of claim 84, wherein said protein comprises a humanized variable heavy chain having the amino acid sequence designated $HumB3V_H$ in Figure 11.

86. The protein of claim 84, wherein said protein comprises a humanized variable heavy chain having the amino acid sequence designated $HumB3V_L$ in Figure 11.

87. The protein of claim 84, wherein said protein comprises a humanized variable heavy chain having the amino acid sequence designated $HumB3V_H$ in Figure 11 and a humanized variable light chain having the amino acid sequence designated $HumB3V_L$ in Figure 11.

88. The protein of claim 84, wherein said protein comprises a humanized variable heavy chain having the amino acid sequence designated $HumB3V_H$ in Figure 11 with the exception that there is a variable heavy chain is back arginine at a position in the variable heavy chain designated as 82b in Figure 11.

89. A pharmaceutical composition comprising a recombinantly produced single chain fusion protein in a concentration sufficient to inhibit tumor growth, together with a pharmaceutically acceptable carrier wherein said fusion protein comprises:

a) a humanized Fv region of both a light and a heavy chain of an antibody wherein said antibody is a monoclonal antibody selected from the group consisting of B1, B3 and B5; and

b) an effector molecule;

wherein both of said Fv regions and said effector molecule are recombinantly fused to form a single molecule that has the binding specificity of a monoclonal antibody selected from the group consisting of B1, B3 and B5.

90. The composition of claim 89, wherein said effector molecule is a *Pseudomonas* exotoxin.

91. The composition of claim 90, wherein said effector molecule is selected from the group consisting of PE38, PE40, PE38KDEL, PE38REDL.

92. The composition of claim 89, wherein said humanized Fv region is a humanized B3(Fv) region.

93. A method of detecting the presence or absence of a cell bearing a $Lewis^Y$ carbohydrate antigen in a patient, said method comprising the steps of:

a) removing a tissue or fluid sample from said patient;

b) adding an antibody to said sample wherein said antibody comprises:

i) a humanized Fv region of both a light and a heavy chain of an antibody wherein said antibody is a monoclonal antibody selected from the group consisting of B1, B3 and B5; and

ii) an effector molecule;

further wherein said Fv regions are recombinantly fused to form a single molecule that has the binding specificity of a monoclonal antibody selected from the group consisting of B1, B3 and B5; and

c) detecting for the presence or absence of a binding complex between the antibody and the antigen.

94. A method of killing or inhibiting the growth of cells bearing a Lewis^Y antigen in a patient, said method comprising administering to the patient a pharmaceutical composition in an amount sufficient to kill or inhibit the growth of said cells, said composition comprising:

a) a humanized Fv region of both a light and a heavy chain of an antibody wherein said antibody is a monoclonal antibody selected from the group consisting of B1, B3 and B5; and

b) an effector molecule;

wherein both of said Fv regions and said effector molecule are recombinantly fused to form a single molecule that has the binding specificity of a monoclonal antibody selected from the group consisting of B1, B3 and B5.

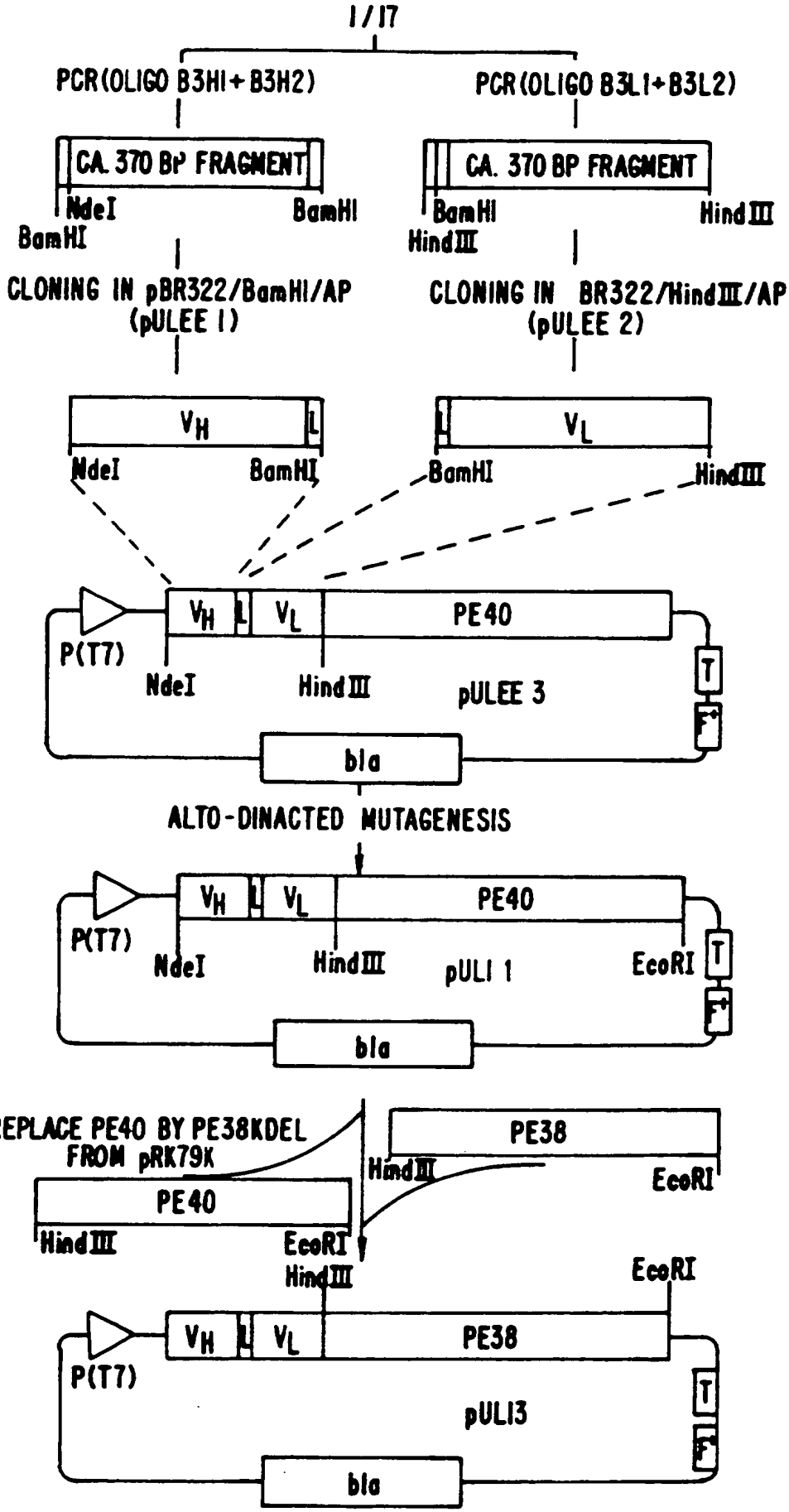

PCR(OLIGO B3H1+B3H2)
PCR(OLIGO B3L1+B3L2)
CA. 370 BP FRAGMENT
NdeI
BamHI
BamHI
CA. 370 BP FRAGMENT
BamHI
HindIII
HindIII
CLONING IN pBR322/BamHI/AP
(pULEE 1)
CLONING IN BR322/HindIII/AP
(pULEE 2)
VH
L
NdeI
BamHI
L
VL
BamHI
HindIII
P(T7)
VH
L
VL
PE40
NdeI
HindIII
pULEE 3
T
F1
bla
ALTO-DINACTED MUTAGENESIS
P(T7)
VH
L
VL
PE40
NdeI
HindIII
pULI 1
EcoRI
T
F1
bla
REPLACE PE40 BY PE38KDEL
FROM pRK79K
PE40
HindIII
EcoRI
PE38
HindIII
EcoRI
HindIII
EcoRI
P(T7)
VH
L
VL
PE38
pULI3
T
F1
bla

FIG. 1A.

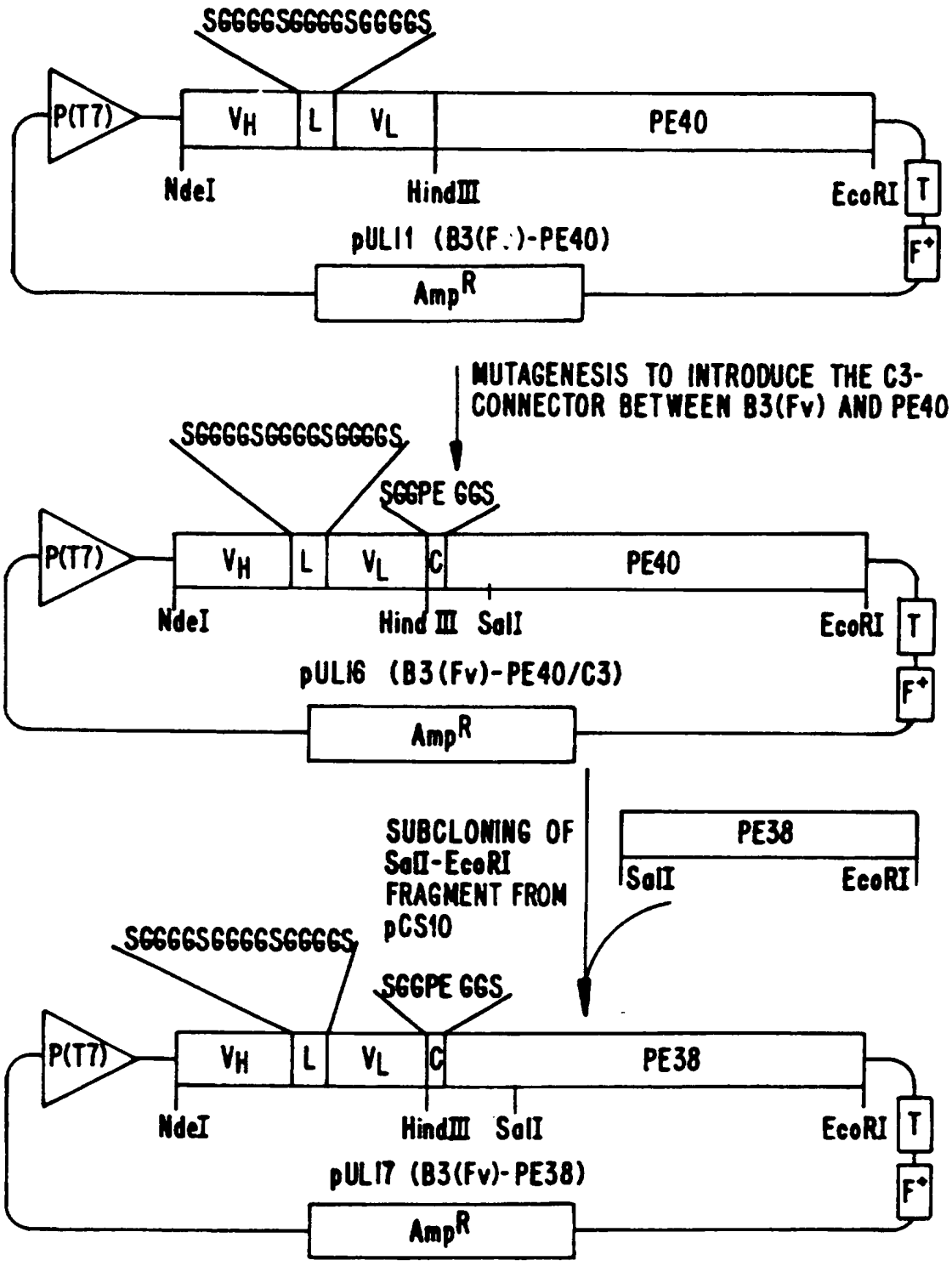

SGGGGSGGGGSGGGGS
P(T7)
VH
L
VL
PE40
NdeI
HindIII
EcoRI
T
F+
pUL11 (B3(Fv)-PE40)
AmpR
MUTAGENESIS TO INTRODUCE THE C3-CONNECTOR BETWEEN B3(Fv) AND PE40
SGGGGSGGGGSGGGGS
SGGPE GGS
P(T7)
VH
L
VL
C
PE40
NdeI
Hind III
SalI
EcoRI
T
F+
pUL16 (B3(Fv)-PE40/C3)
AmpR
SUBCLONING OF SalI-EcoRI FRAGMENT FROM pCS10
PE38
SalI
EcoRI
SGGGGSGGGGSGGGGS
SGGPE GGS
P(T7)
VH
L
VL
C
PE38
NdeI
HindIII
SalI
EcoRI
T
F+
pUL17 (B3(Fv)-PE38)
AmpR

FIG. 1B.

```
                                  NdeI |----Fv HEAVY CHAIN--
  1 TTTAACTTTAAGAAGGAGATATACATATGGATGTGAAGCTGGTGGAGTCT  50
                    SD          M  D  V  K  L  V  E  S
                                   E  V  K  L  V  E  S
    --------->
 51 GGGGGAGGCTTAGTGCAGCCTGGAGGGTCCCTGAAACTCTCCTGTGCAAC 100
    G  G  G  L  V  Q  P  G  G  S  L  K  L  S  C  A  T
    G  G  G  L  V  Q  P  G  G  S  L

101 CTCTGGATTCACTTTCAGTGACTATTACATGTATTGGGTTCGCCAGACTC 150
     S  G  F  T  F  S  D  Y  Y  M  Y  W  V  R  Q  T  P

151 CAGAGAAGAGGCTGGAGTGGGTCGCATACATTAGTAATGATGATAGTTCC 200
      E  K  R  L  E  W  V  A  Y  I  S  N  D  D  S  S

201 GCCGCTTATTCAGACACTGTAAAGGGCCGGTTCACCATCTCCAGAGACAA 250
    A  A  Y  S  D  T  V  K  G  R  F  T  I  S  R  D  N

251 TGCCAGGAACACCCTCTACCTGCAAATGAGCCGTCTGAAGTCTGAGGACA 300
     A  R  N  T  L  Y  L  Q  M  S  R  L  K  S  E  D  T

301 CAGCCATATATTCCTGTGCAAGAGGACTGGCCTGGGGAGCCTGGTTTGCT 350
      A  I  Y  S  C  A  R  G  L  A  W  G  A  W  F  A
                                                  BamHI
351 TACTGGGGCCAAGGGACTCTGGTCACTGTCTCCTCAGGCGGAGGCGGATC 400
    Y  W  G  Q  G  T  L  V  T  V  S  S  G  G  G  G  S
             <-----Fv HEAVY CHAIN--|----------LINKER-

    ---------LINKER---------------|--Fv LIGHT CHAIN---
401 CGGTGGTGGCGGATCTGGAGGTGGCGGAAGCGATGTGCTGATGACCCAGT 450
     G  G  G  G  S  G  G  G  G  S  D  V  L  M  T  Q  S
                                   D  V  L  M  T  Q  S

    -----Fv LIGHT CHAIN---------->
451 CTCCATTGAGTTTACCTGTCAGTCTTGGAGATCAAGCCTCCATCTCTTGC 500
      P  L  S  L  P  V  S  L  G  D  Q  A  S  I  S  C
      P  L  S  L  P  V  S  L  G  ?  Q

501 AGATCTAGTCAGATCATTGTACATAGTAATGGAAACACCTATTTAGAATG 550
    R  S  S  Q  I  I  V  M  S  N  G  N  T  Y  L  E  W

551 GTACCTGCAGAAACCAGGCCAGTCTCCAAAGCTCCTGATCTACAAAGTTT 600
     Y  L  Q  K  P  G  Q  S  P  K  L  L  I  Y  K  V  S
```

FIG. 2A-1.

```
601 CCAACCGATTTTCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGG 650
      N  R  F  S  G  V  P  D  R  F  S  G  S  G  S  G

651 ACAGATTTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGGAGT 700
    T  D  F  T  L  K  I  S  R  V  E  A  E  D  L  G  V

701 TTATTACTGCTTTCAAGGTTCACATGTTCCATTCACGTTCGGCTCGGGGA 750
     Y  Y  C  F  Q  G  S  N  V  P  F  T  F  G  S  G  T

                    HindIII
751 CAAAGCTGGAAATTAAAGCTTT.....                        772
      K  L  E  I  K  A  F -> PE40
```

FIG. 2A-2.

```
721 CACATGTTCCATTCACGTTCGGCTCGGGGACAAAGCTGGAAATTAAATAA 770
      H  V  P  F  T  F  G  S  G  T  K  L  E  I  K  *

    EcoRI
771 TGAATTCC..                                        779
    * -> TERM
```

FIG. 2B.

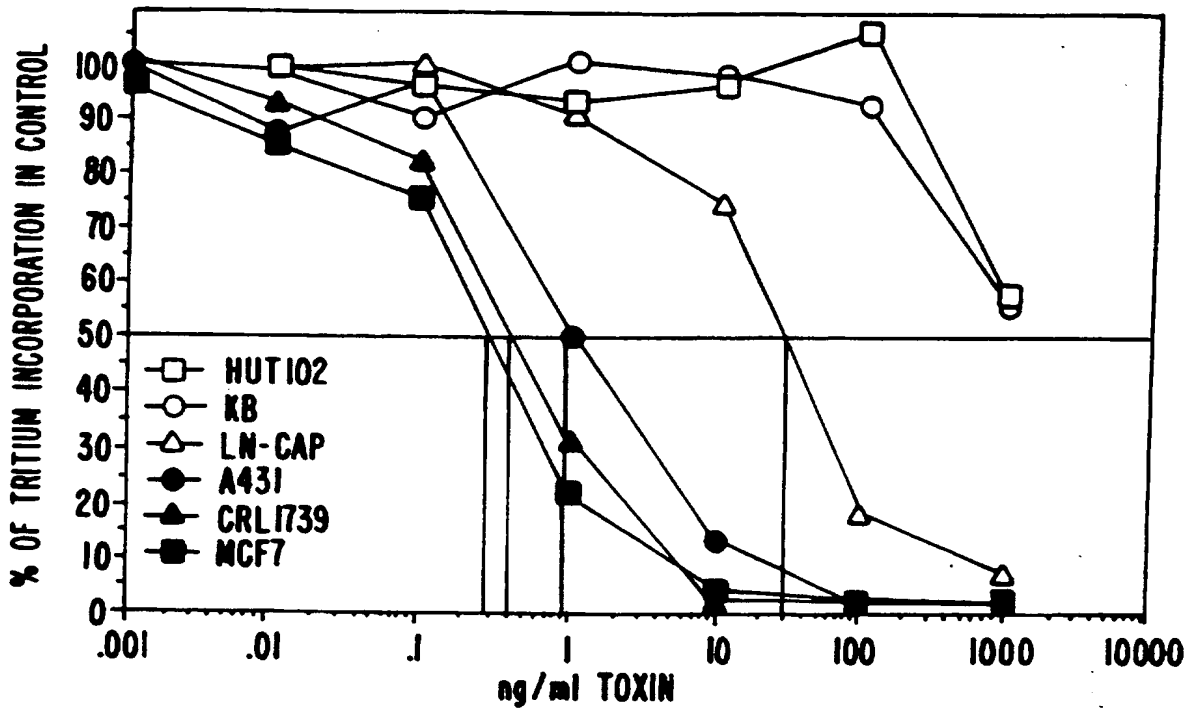

% OF TRITIUM INCORPORATION IN CONTROL
100
90
80
70
60
50
40
30
20
10
0
HUT102
KB
LN-CAP
A431
CRL1739
MCF7
.001
.01
.1
1
10
100
1000
10000
ng/ml TOXIN

FIG. 3A.

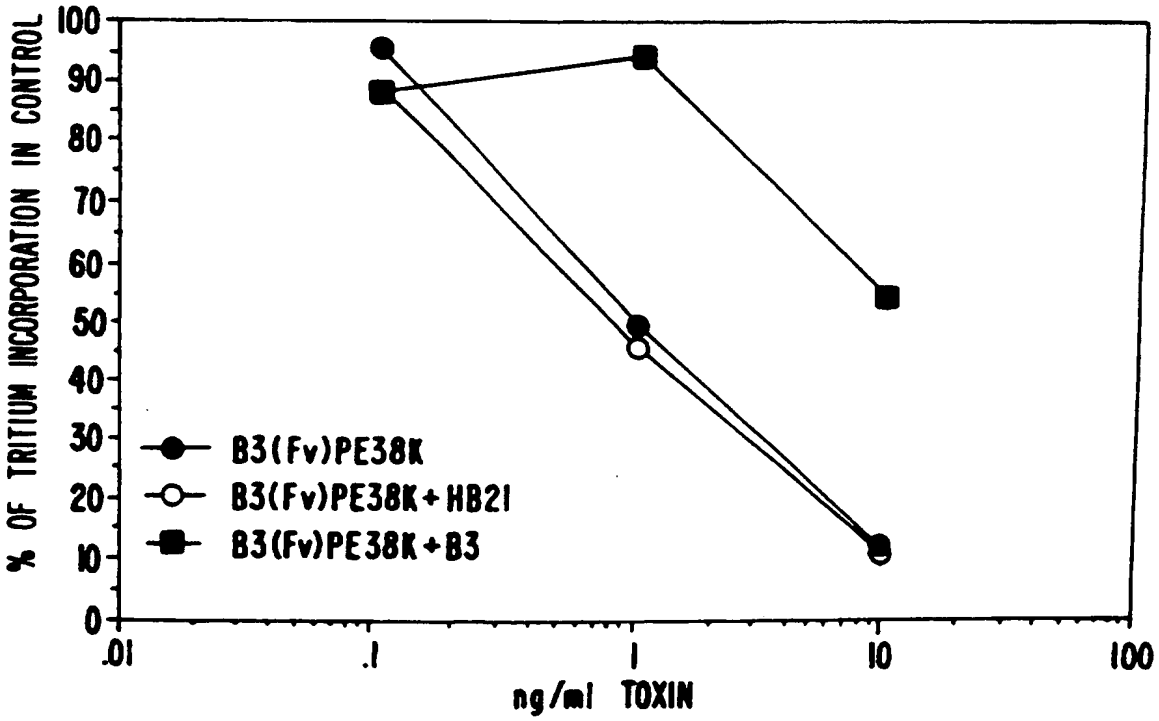

% OF TRITIUM INCORPORATION IN CONTROL
100
90
80
70
60
50
40
30
20
10
0
B3(Fv)PE38K
B3(Fv)PE38K+HB21
B3(Fv)PE38K+B3
.01
.1
1
10
100
ng/ml TOXIN

FIG. 3B.

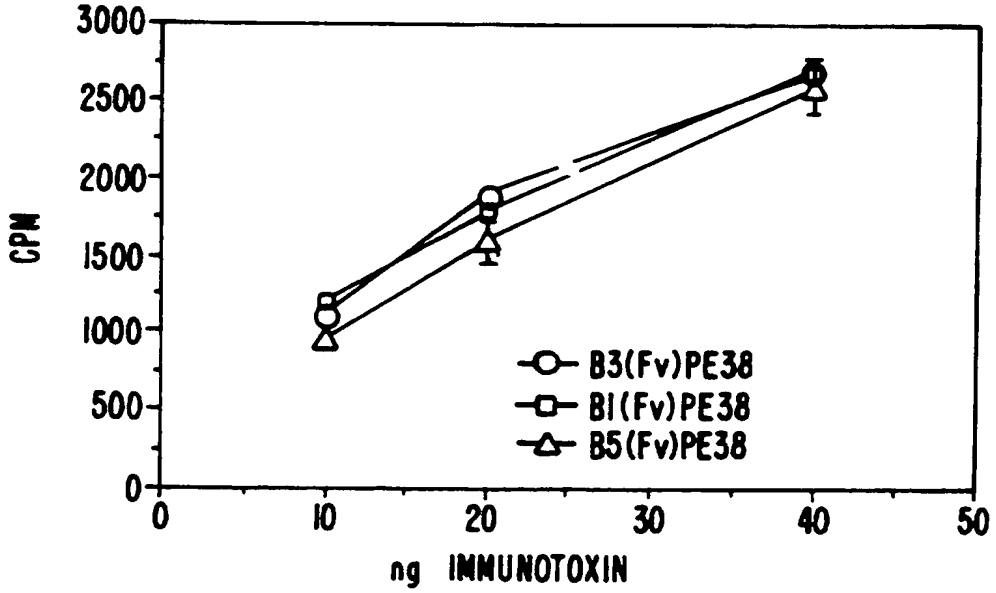

3000
2500
2000
1500
1000
500
0
CPM
0
10
20
30
40
50
ng IMMUNOTOXIN
B3(Fv)PE38
BI(Fv)PE38
B5(Fv)PE38

FIG. 4A.

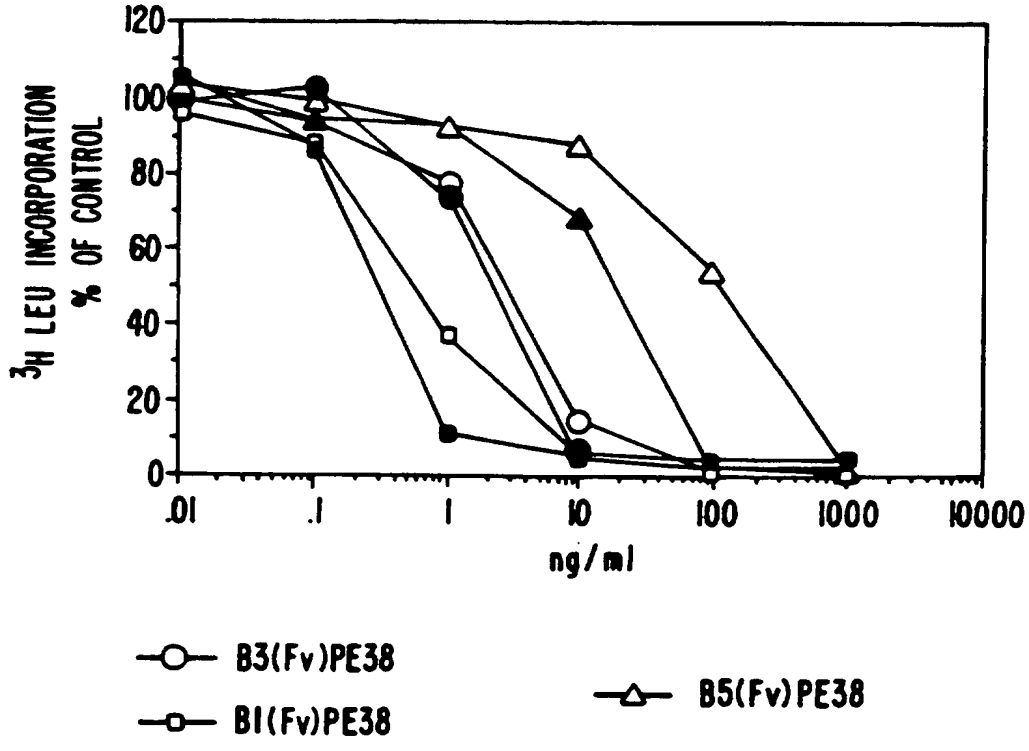

120
100
80
60
40
20
0
^{3}H LEU INCORPORATION
% OF CONTROL
.01
.1
1
10
100
1000
10000
ng/ml
B3(Fv)PE38
BI(Fv)PE38
B5(Fv)PE38

FIG. 4B.

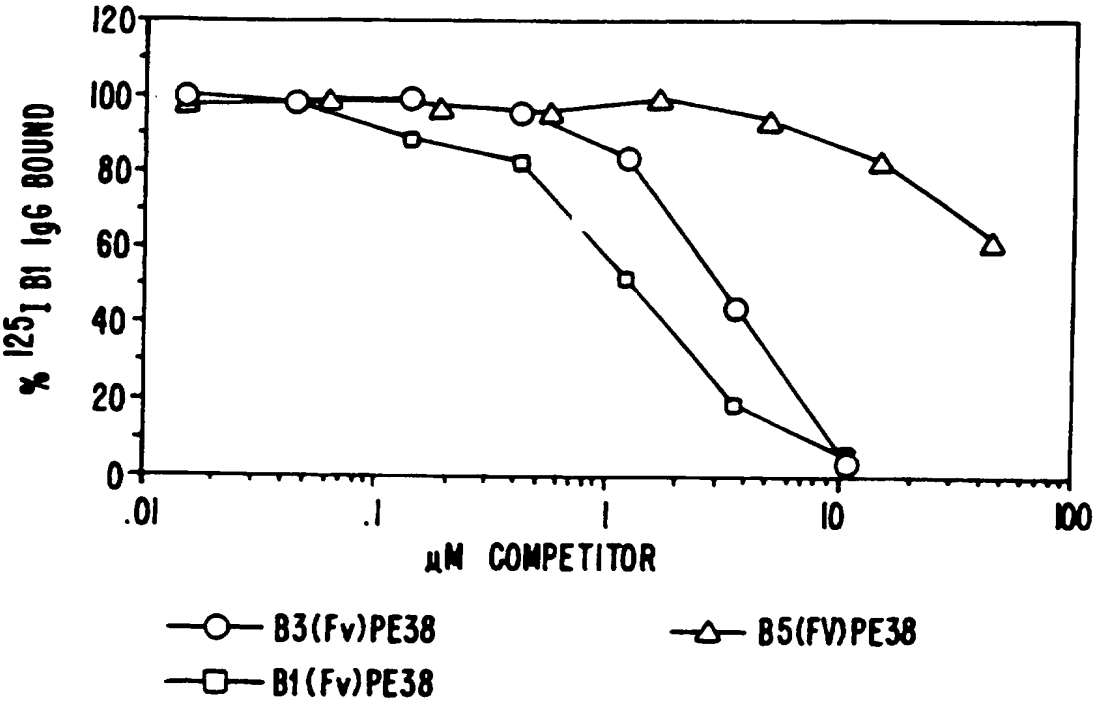


FIG. 5A.

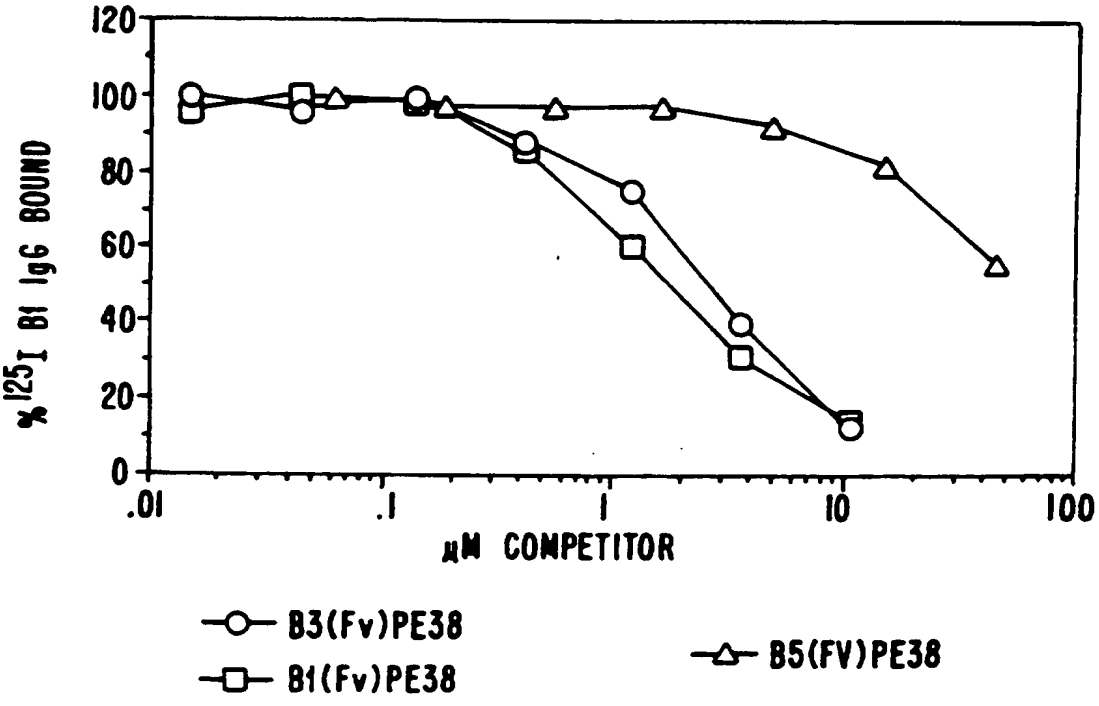


FIG. 5B.

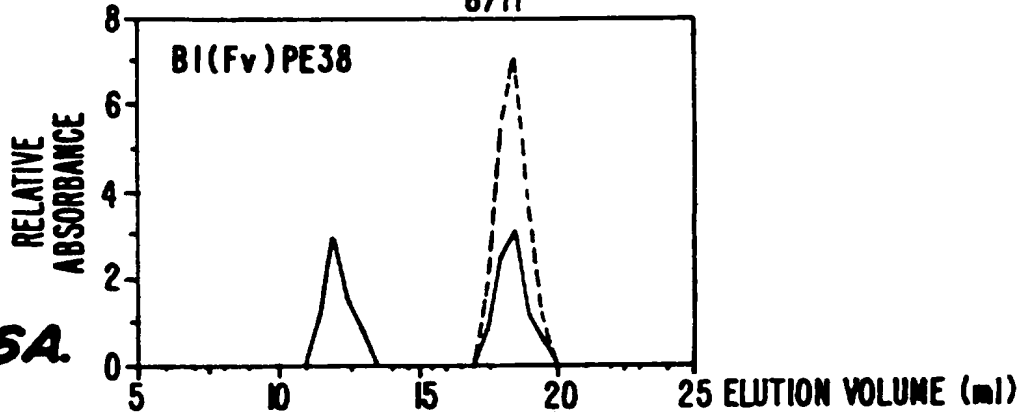


FIG. 6A.

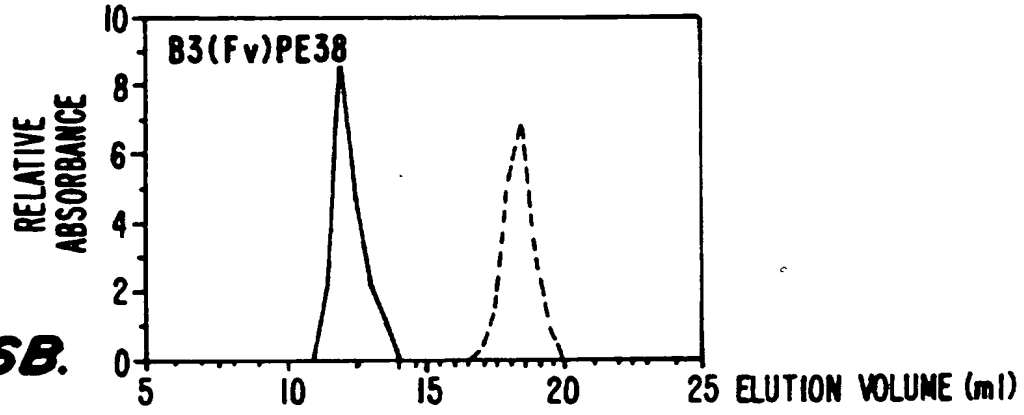


FIG. 6B.

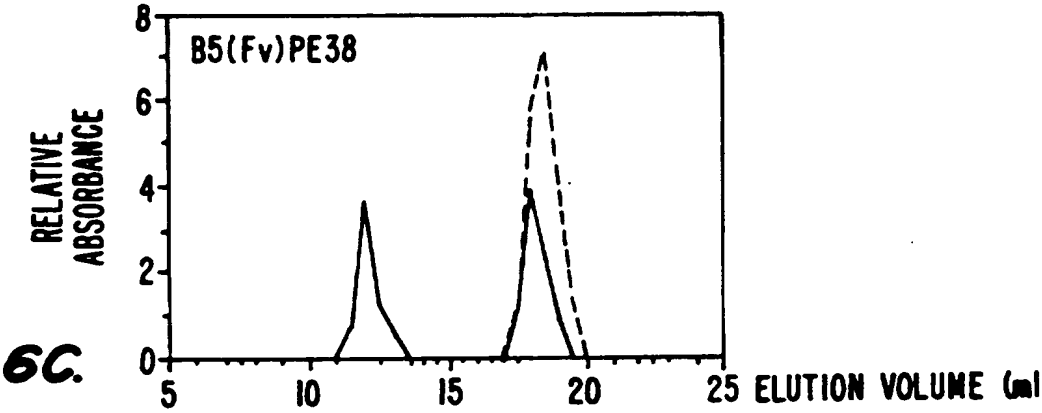


FIG. 6C.

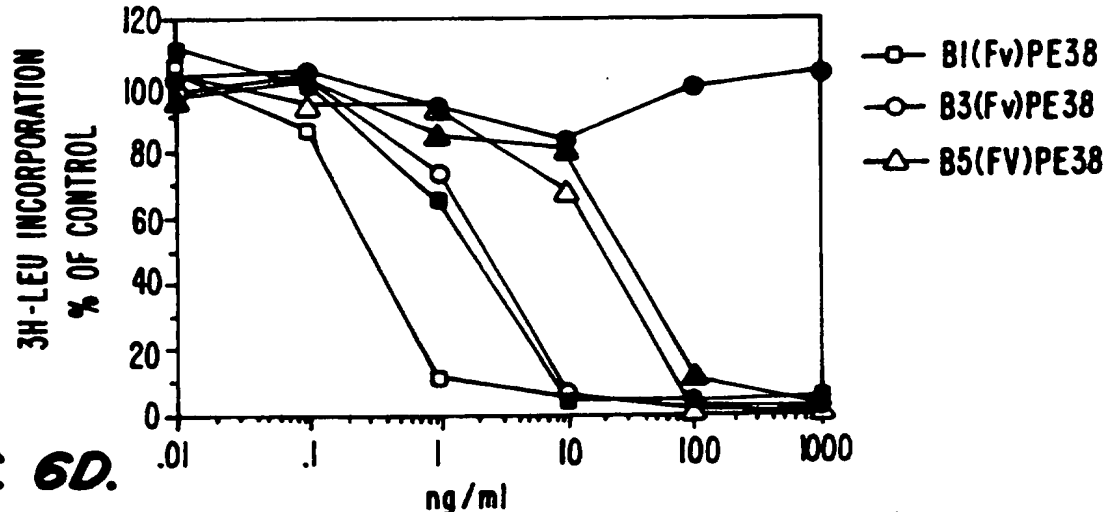


FIG. 6D.

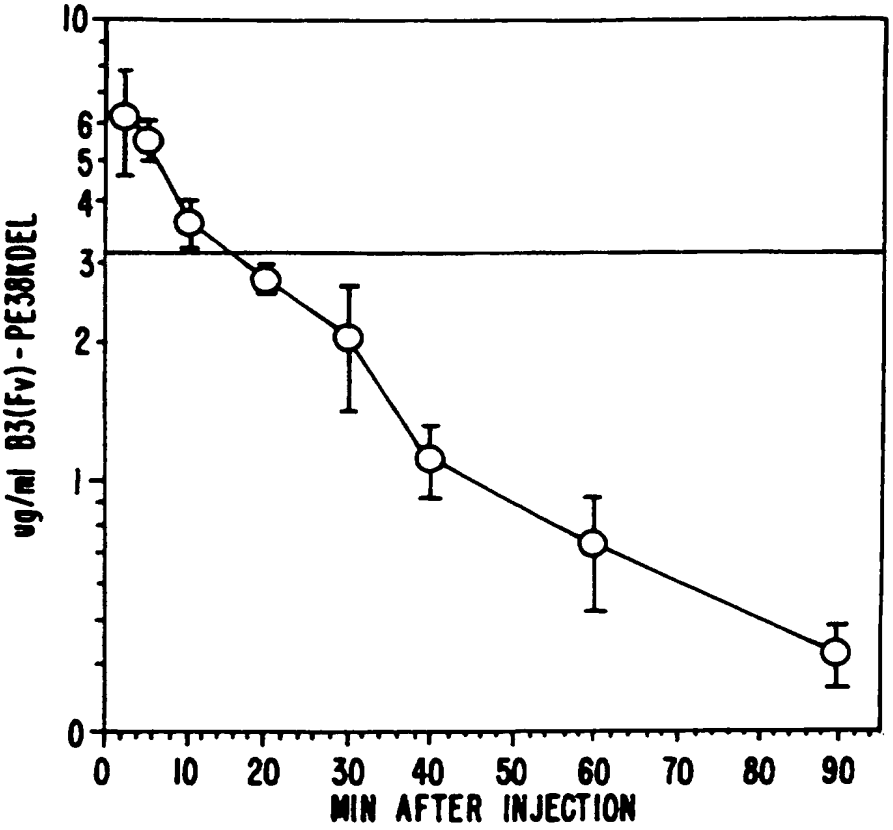


FIG. 7.

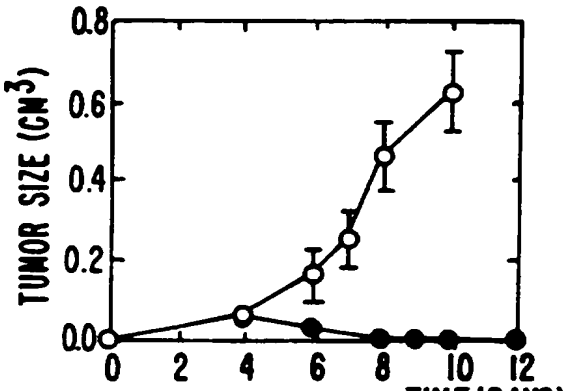


FIG. 8A.

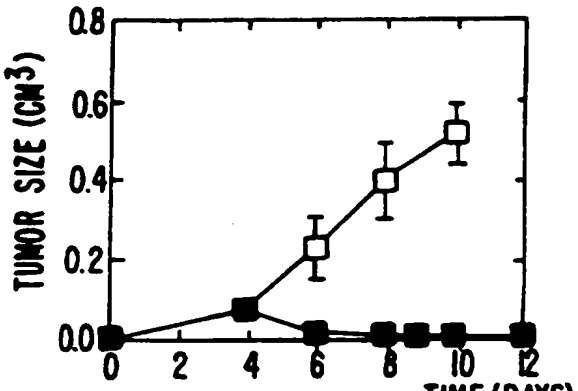


FIG. 8B.

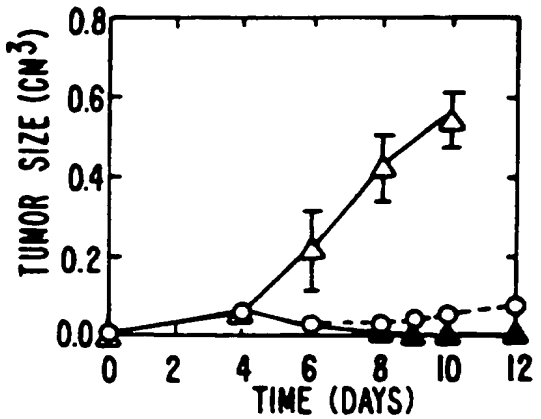


FIG. 8C.

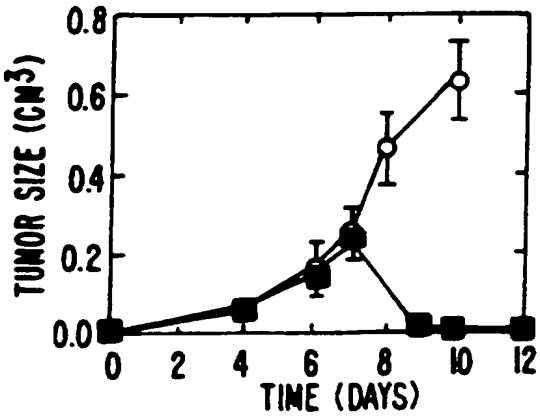


FIG. 8D.

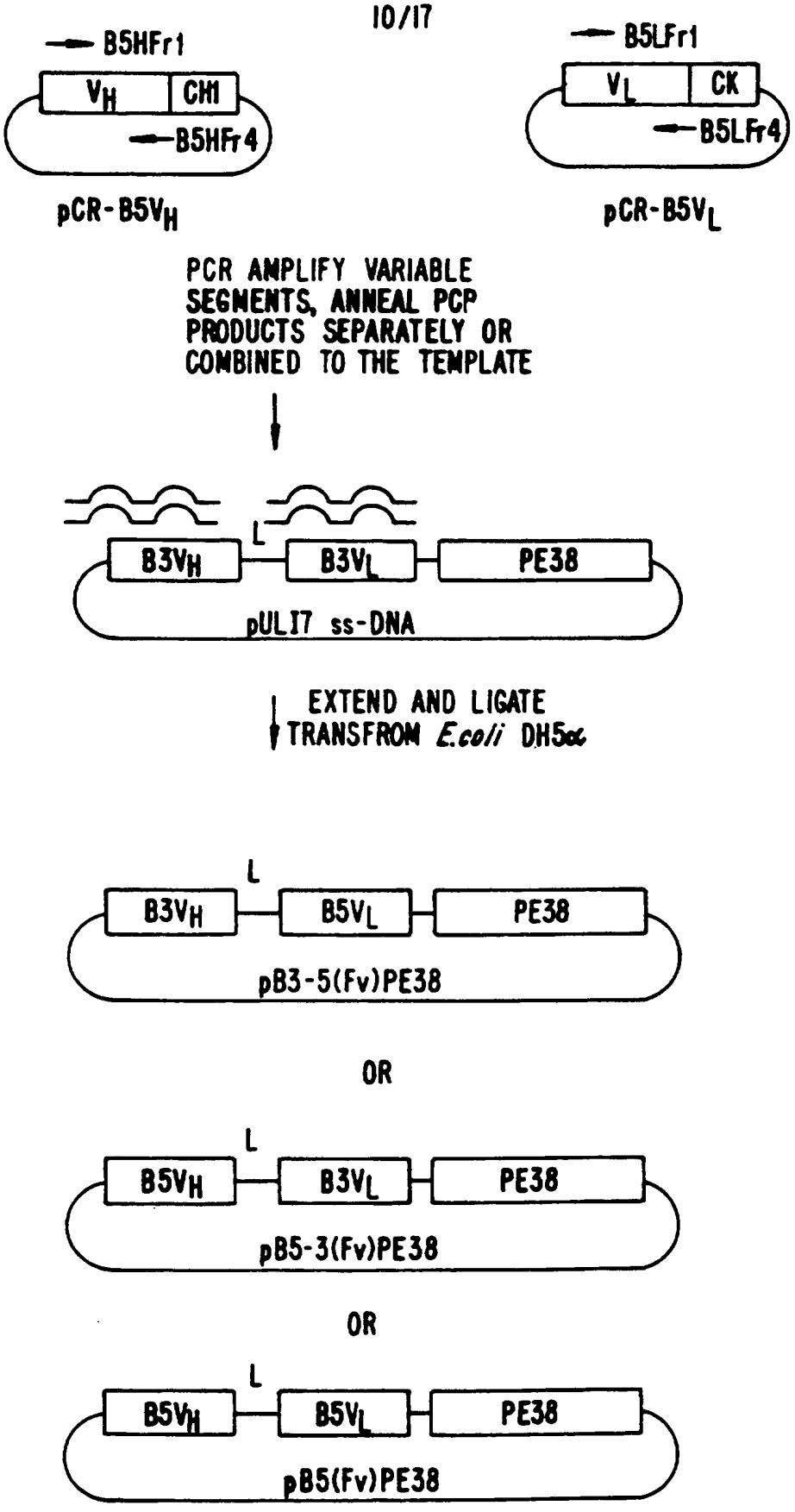


FIG. 9.

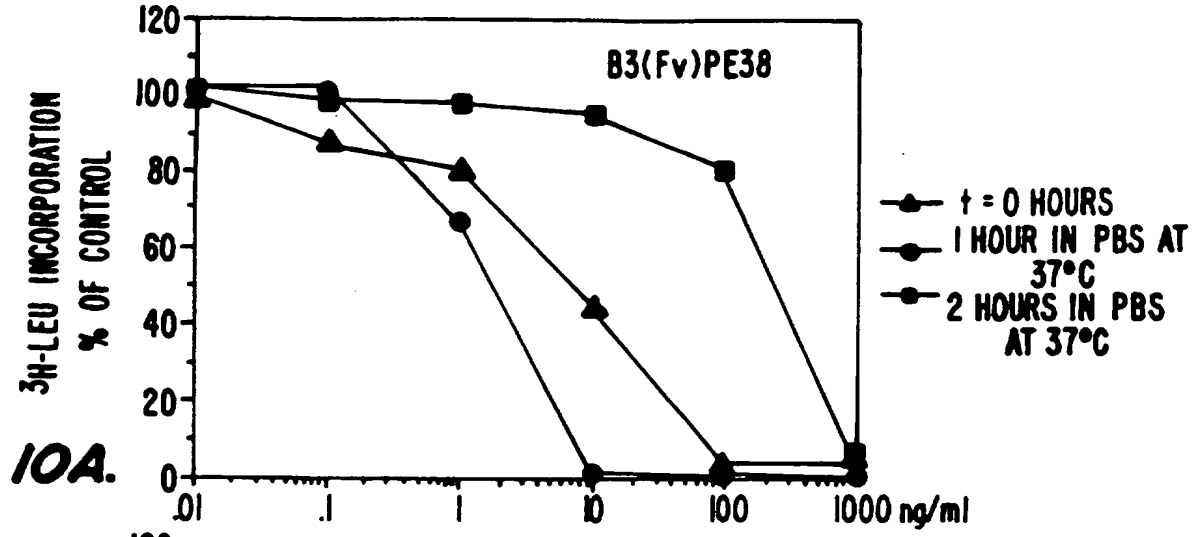


FIG. 10A.

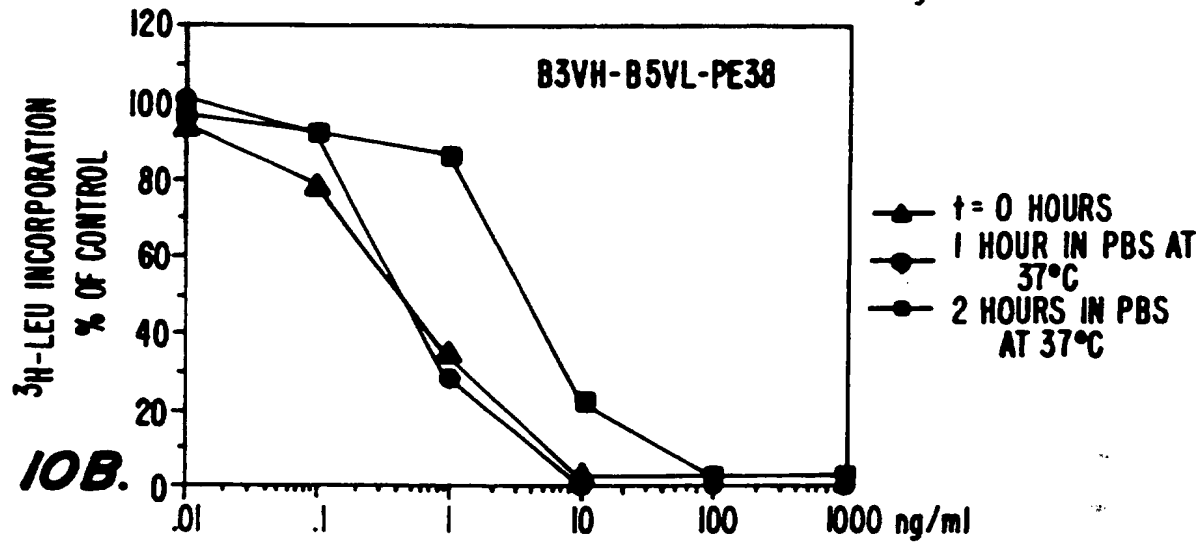


FIG. 10B.

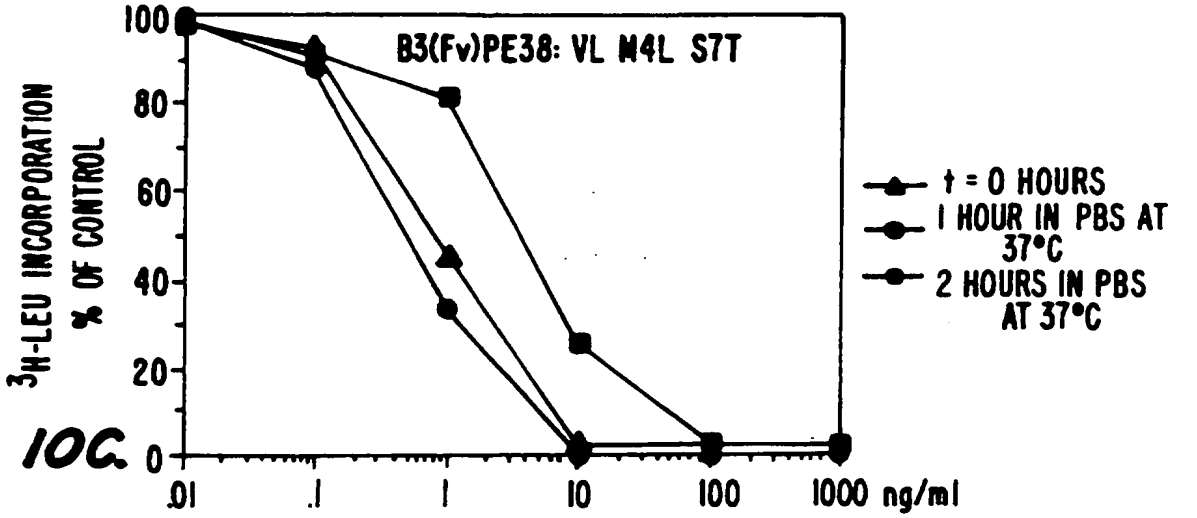


FIG. 10C.

```
                  11   16                  40 42 44
         | |       |    |   |    |     CDR1      |  /  /        CDR2
B3VH     DVKLVESGGGLVQPGGSLKLSCATSGFTFS DYYMY WVRQTPEKRLEWVA YISNDDSSAAYSDTVKG
56P1'CL  QVELVESGGGVVQPGRSLRLSCAASGFTFS SYAMH WVRQAPGKGLEWVA VISYDGSNKYYADSVKG
HumB3VH  DVKLVESGGGVVQPGRSLKLSCATSGFTFS DYYMY WVRQAPGKGLEWVA YISNDDSSAAYSDRVKG
         * *               *    *
```

```
            74 75  82a 82b 83 84
             \ |    \  |  / /      | |     CDR3
B3VH     RFTISTDNARNTLYLQMSRLKSEDRAIYSCAR GLAWGAWFAY WGQGTLVTVSS
56P1'CL  RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR RSARTYYFDY WGQGTLVTVSS
HumB3VH  RFTISRDNSKNTLYLQMNSLRAEDTAIYSCAR TLAWTAWRAY WGQGTLVTVSS
                                  * *
```

FIG. 11A.

```
                   14 15 17 18                                45
          ||        \  | /  /          CDR1               |    |    CDR2
B3VL     DVLMTQSPLSLPVSLGDQASISC RSSQIIVHSNGNTYLE WYLQKPGQSPKLLIY KVSNRFS
GM607    DIVMTQSPLSLPVTPGEPASISC RSSQSLLHSNGYNYLD WYLQKPQQSPQLLIY LGSNRAS
HumB3VL  DVLMTQSPLSLPVTPGEPASISC RSSQIIVHSNGNTYLE WYLQKPGQSPQLLIY KVSNRFS
          **                                            *
```

```
                                 83              100 104
                                  |      CDR3      |   |
B3VL     GVPDRFSGSGSGTDFTLKISRVEAEDLGVYYC FQFSHVPFT FGSGTKLEIK
GM607    GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC MQGLQTPQT FGQGTKVEIK
HumB3VL  GVPDRFSGSGSGTDFRLKISRVEAEDVGVYYC FQFSHVPFT FGQGTKVEIK
```

FIG. 11B.

FIG. 12A.

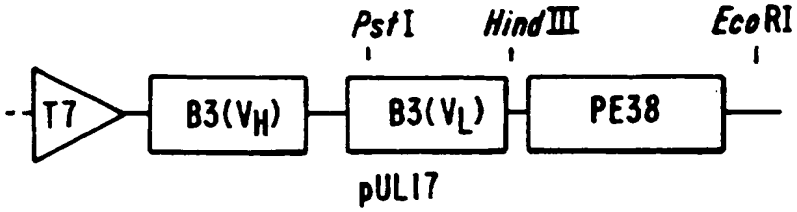


FIG. 12B.

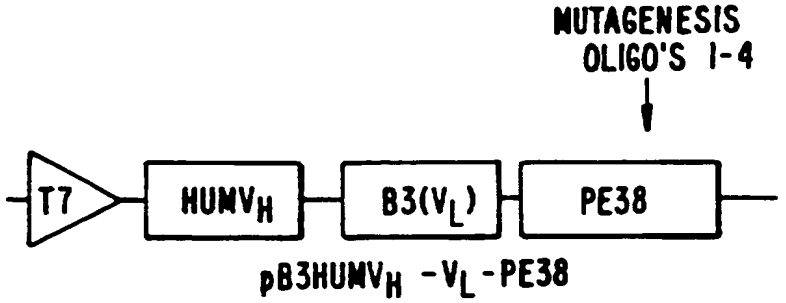


FIG. 12C.

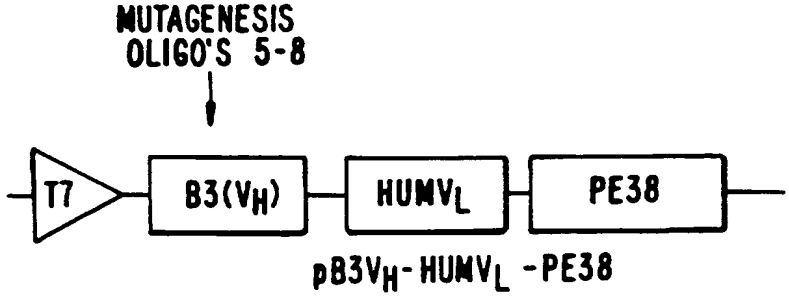
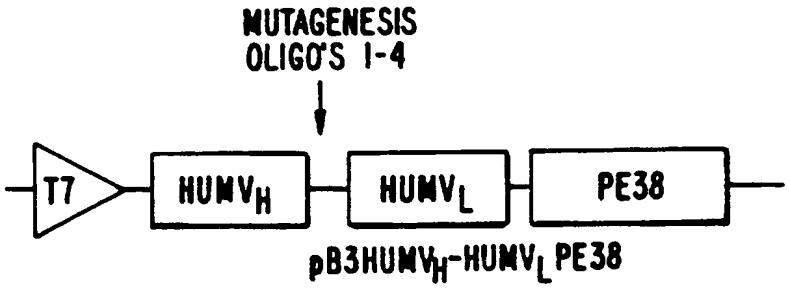


FIG. 12D.



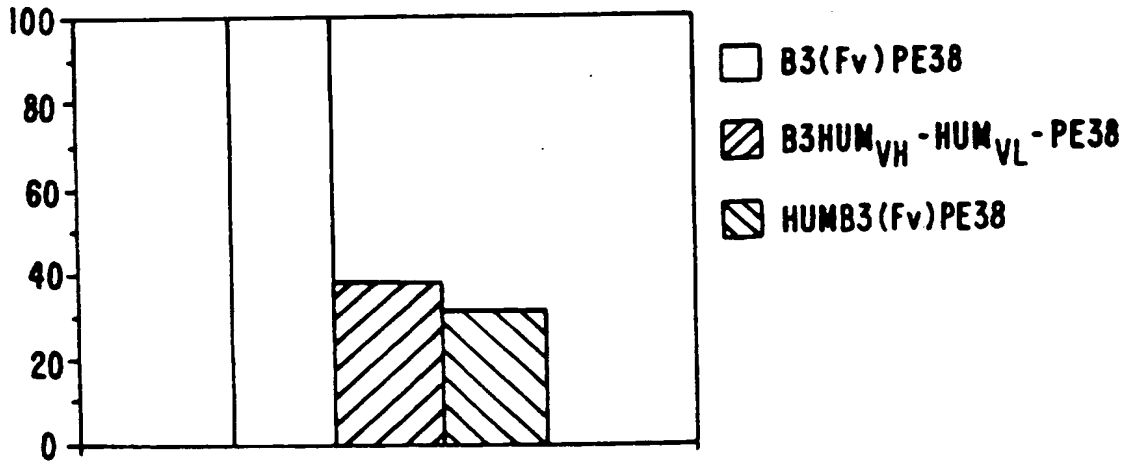


FIG. 14.

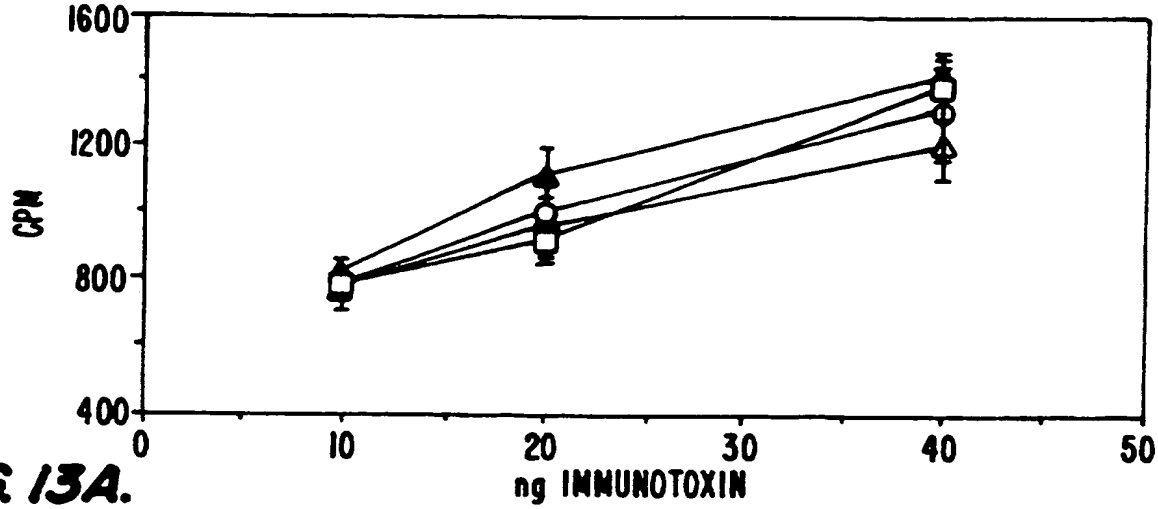


FIG. 13A.

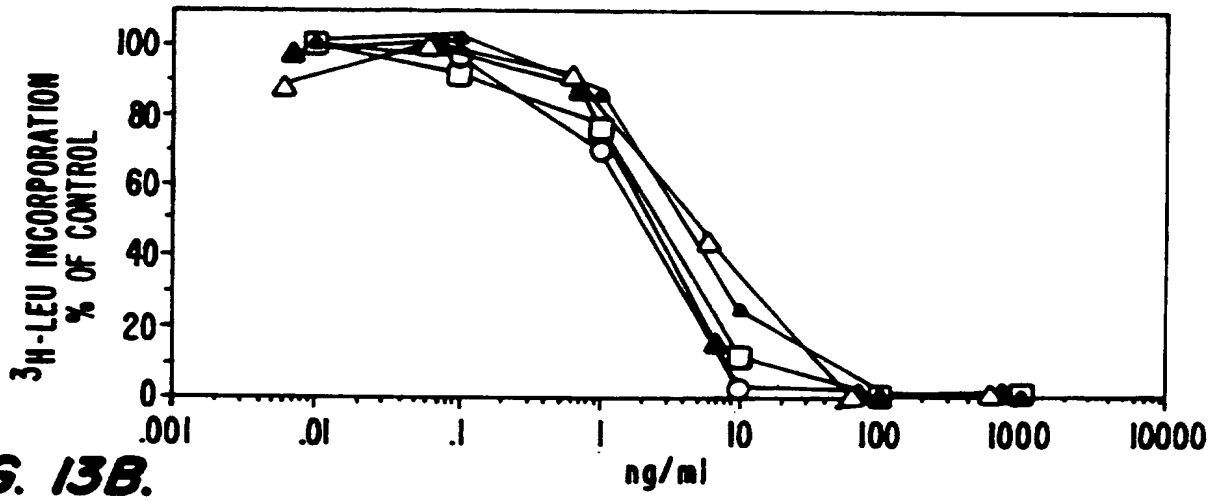


FIG. 13B.

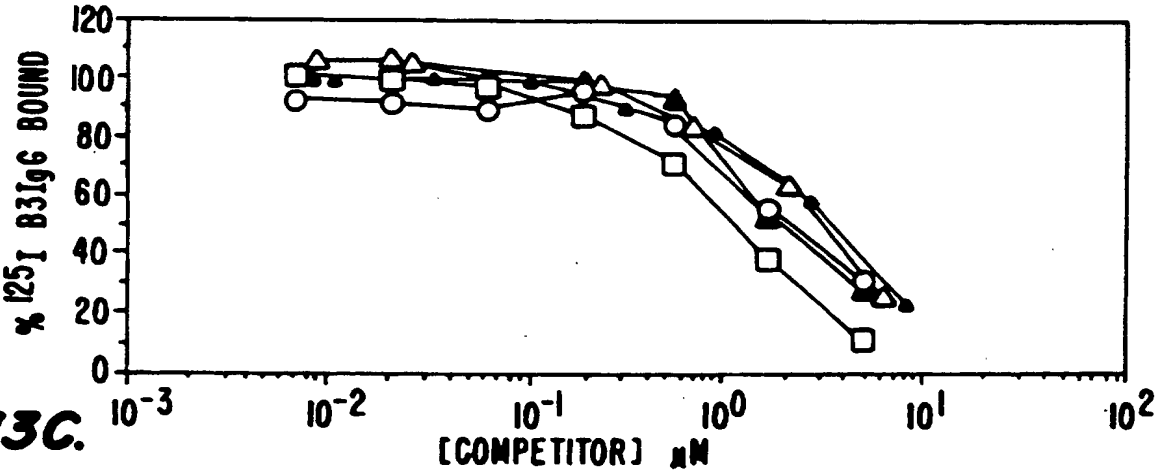


FIG. 13C.

```
GAGGTGCAGCTGGTGGAATCTGGAGGAGGCTTAGTGAAGCCTGGAGGGTCCCTGAAA
 E  V  Q  L  V  E  S  G  G  G  L  V  K  P  G  G  S  L  K
 E  V  Q  L  V  E  S  G  G  G  L  V  K  P  G  G  S  L
CTCTCCTGTGCAGCCTCTGGATTCATTTTCAGTGACAATTACATGTATTGGGTTCGC
 L  S  C  A  A  S  G  F  I  F  S  D  N  Y  M  Y  W  V  R
                                   CDR 1
CAGACTCCGGAGAAGAGGCTGGAGTGGGTCGCAACCATTAGTGATGGTGGCACTTAT
 Q  T  P  E  K  R  L  E  W  V  A  T  I  S  D  G  G  T  Y
                                                CDR 2
ATCGACTATTCAGACAGTGTGAAGGGGCGATTCACCATCTCCAGAGACAATGCCAAG
 I  D  Y  S  D  S  V  K  G  R  F  T  I  S  R  D  N  A  K

AATAATCTGTACTTGCAAATGAGCAGTCTGAGGTCTGAGGACACAGGCATGTATTAT
 N  N  L  Y  L  Q  M  S  S  L  R  S  E  D  T  G  M  Y  Y

TGTGGAAGGAGTCCGATCTACTATGATTACGCCCCGTTTACTTACTGGGGCCAAGGG
 C  G  R  S  P  I  Y  Y  D  Y  A  P  F  T  Y  W  G  Q  G
                CDR 3
ACTCTGGTCACTGTCTCTGCAGCCAAAACGACACCCCCATCTGTCTATCCACTGGCC
 C  L  V  T  V  S  A  A  K  T  T  P  P  S  V  Y  P  L  A

CCTGGATCTGCT
 P  G  S  A
```

FIG. 15A.

```
GATGTTGTGATGACCCAGACTCCACTCTCCCTGCCTGTCAGTCTTGGAGATCAAGCC
 D  V  V  M  T  Q  T  P  L  S  L  P  V  S  L  G  D  Q  A
 D  V  V  M  T  Q  T  P  L  S  L  P  V  S  L  G  D
TCCATCTCTTGCAGATCTAGTCAAAACCTTGTACACAGTGATGGAAAAACCTATTTA
 S  I  S  C  R  S  S  Q  N  L  V  H  S  D  G  K  T  Y  L
                                   CDR 1
CATTGGTTCCTGCAGAAGCCTGGCCAGTCTCCAACGCTCCTGATCTACAAAGTTTCC
 H  W  F  L  Q  K  P  G  Q  S  P  T  L  L  I  Y  K  V  S
                                                CDR 2
AACCGATTTTCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGATTTC
 N  R  F  S  G  V  P  D  R  F  S  G  S  G  S  G  T  D  F

ATACTCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTTATTTCTGCTCTCAA
 I  L  K  I  S  R  V  E  A  E  D  L  G  V  Y  F  C  S  Q

AGTACACATGTTCCGCTCACGTTCGGTGCTGGGACCAAGCTGGAGCTGAAACGGGCT
 S  T  H  V  P  L  T  F  G  A  G  T  K  L  E  L  K  R  A
      CDR 3
GATGCTGCACCAACTGTATCCATCTTCCCACCA
 D  A  A  P  T  V  S  I  F  P  P
```

FIG. 15B.

```
GAGGTGAAGCTGGTGGAATCTGGAGGAGGCTTAGTGCAGCCTGGAGGGTCCCTGAAA
 E  V  K  L  V  E  S  G  G  G  L  V  Q  P  G  G  S  L  K
 E  V  K  L  V  E  S  G  G  G  L  V  Q  P  G
CTCTCCTGTGCAACCTCTGGATTTACTTTCAGTGACTATTACATGTATTGGGTTCGC
 L  S  C  A  T  S  G  F  T  F  S  D  Y  Y  M  Y  W  V  R
                                   CDR 1
CAGACTCCAGAGAAGAGGCTGGAGTGGGTCGCATACATTAGTAATGGTGGTGGTAGC
 Q  T  P  E  K  R  L  E  W  V  A  Y  I  S  N  G  G  G  S
                                                CDR 2
ACCTATTATCCAGACACTGTAAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAG
 T  Y  Y  P  D  T  V  K  G  R  F  T  I  D  R  D  N  A  K

AACACCCTGTACCTGCAGATGAGCCGTCTGAAGTCTGAGGACACAGCCATGTATTAC
 N  T  L  Y  L  Q  M  S  R  L  K  S  E  D  T  A  M  Y  Y

TGTGCAAGGGGGCTCTCTGATGGTTCCTGGTTTGCTTACTGGGGCCAAGGGACTCTG
 C  A  R  G  L  S  D  G  S  W  F  A  Y  W  G  Q  G  T  L
                CDR 3
GTCACTGTCTCCTCAGGCGGAGGCGGATCCGGT
 V  T  V  S  S  G  G  G  G  S  G
```

FIG. 16A.

```
GATGTTTTGTTGACCCAAACTCCACTCTCCCTGCCTGTCAGTCTTGGAGATCAAGCC
 D  V  L  L  T  Q  T  P  L  S  L  P  V  S  L  G  D  Q  A
 D  V  L  L  T  Q  T  P  L  S  L  P  V  S  L
TCTATTTCTTGTAGATCTAGTCAGAGCATTGTACATAGTAATGGAAACACCTATTTA
 S  I  S  C  R  S  S  Q  S  I  V  H  S  N  G  N  T  Y  L
                                   CDR 1
GAATGGTACCTGCAGAAACCAGGCCAGTCTCCAAAGCTCCTGATCTACAAAGTTTCC
 E  W  Y  L  Q  K  P  G  Q  S  P  K  L  L  I  Y  K  V  S
                                                CDR 2
AACCGATTTTCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGATTTC
 N  R  F  S  G  V  P  D  R  F  S  G  S  G  S  G  T  D  F

ACACTCAAGATCAGCAGAGTGGAGGCTFAGGATCTGGGAGTTTATTACTGCTTTCAA
 T  L  K  I  S  R  V  E  A  E  D  L  G  V  Y  Y  C  F  Q

GGTTCACATGTTCCATTCACGTTCGGCTCGGGGACAAAGTTGGAAATAAAGCGGGCT
 G  S  H  V  P  F  T  F  G  S  G  T  K  L  E  I  K  R  A
     CDR 3
GATGCTGCACCAACTGTATCCATCTTCCCACCA
 D  A  A  P  T  V  S  I  F  P  P
```

FIG. 16B.

VH:
DVKLVESGGGLVQPGGSLKLSCATSGFTFS (DYYMY) WVRQTPEKRLEWVA (YISNDDSSAAYSDTVKG)
RFTISTDNARNTLYLQMSRLKSEDTAIYSCAR (GLAWGAWFAY) WGQGTLVTVSS

LINKER:
GGGGSGGGGSGGGGS

VL:
DVLMTQSPLSLPVSLGDQASISC (RSSQIIVHSNGNTYLE) WYLQKPGQSPKLLIY (KVSNRFS)
GVPDRFSFSFSFTDFTLKISRVEAEDLGVYYC (FQGSHVPFT) FGSGTKLEIK

FIG. 17.

VH:
GATGTGAAGCTGGTGGAGTCTGGGGGAGGCGTCGTGCAGCCCGGGCGCTCCCTGAAACTCTCCTGTGCAACCTCTG
GATTCACTTTCAGTGACTATTACATGTATTGGGTTCGCCAGGCCCCGGGCAAGGGCCTGGAGTGGGTCGCATACAT
TAGTAATGATGATAGTTCCGCCGCTTATTCAGACACTGTAAAGGGCCGGTTCACCATCTCTAGAGACAA?AGCAAG
AACACCCTCTACCTGCAAATGAACCGTCTGCGCGCCGAGGACACAGCCATATATTCCTGTGCAAGAGGACTGGCCT
GGGGAGCCTGGTTTGCTTACTGGGGCCAAGGGACTCTGGTCACTGTCTCCTCA

LINKER: ggcggaggcggatccggtggtggcggatctggaggtggcggaagc

VL:
GATGTGCTGATGACCCAGTCTCCATTGAGTTTACCTGTCACCCCGGGAGAGCCGGCCTCCATCTCTTGCAGATCTA
GTCAGATCATTGTACATAGTAATGGAAACACCTATTTAGAATGGTACCTGCAGAAACCAGGCCAGTCTCCACAGCT
GCTGATCTACAAAGTTTCCAACCGATTTTCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGATTTC
ACACTCAAGATCAGCAGAGTGGAGGCTGAGGACGTCGGAGTTTATTACTGCTTTCAAGGTTCACATGTTCCATTCA
CGTTCGGCCAGGGTACCAAGGTCGAAATTAAA

FIG. 18.

INTERNATIONAL SEARCH REPORT

International Application No
US 95/13811

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/62 C12N15/13 C07K19/00 C07K16/30 A61K47/42
A61K39/395 G01N33/577 G01N33/574 C07K16/00 C07K16/46
//C07K14/21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,93 07286 (THE UNITED STATES OF AMERICA) 15 April 1993 cited in the application see the whole document ---	1-30
X	BIOCHIMICA ET BIOPHYSICA ACTA, vol. 1198, 1994 AMSTERDAM, THE NETHERLANDS, pages 27-45, XP 000564482 U. BRINKMANN ET AL. 'Immunotoxins against cancer' cited in the application see the whole document ---	1-30
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 February 1996	25 MARCH 1996

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Nooij, F

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 88, no. 19, 1 October 1991 WASHINGTON, DC, USA, pages 8616-8620, U. BRINKMANN ET AL. 'B3(Fv)-PE38KDEL, a single-chain immunotoxin that causes complete regression of a human carcinoma in mice.' cited in the application see abstract see figures 1,2 ---	1-30
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 89, no. 7, 1 April 1992 WASHINGTON, DC, USA, pages 3075-3079, XP 000264181 U. BRINKMANN ET AL. 'Independent domain folding of Pseudomonas exotoxin and single-chain immunotoxins: Influence of interdomain connections.' cited in the application see figure 1 ---	1-22
X	BIOCONJUGATE CHEMISTRY, vol. 5, no. 4, July 1994 WASHINGTON, DC, USA, pages 321-326, XP 000564453 I. BENHAR ET AL. 'Mutations of two lysine residues in the CDR loops of a recombinant immunotoxin that reduce its sensitivity to chemical derivatization.' cited in the application see abstract see figure 1 ---	1-30, 34-37
X	CANCER RESEARCH, vol. 53, no. 2, 15 January 1993 PHILADELPHIA, PA, USA, pages 334-339, P. FRIEDMAN ET AL. 'BR96 sFv-PE40, a potent single-chain immunotoxin that selectively kills carcinoma cells.' see abstract see discussion ---	1-30
X	THE JOURNAL OF IMMUNOLOGY, vol. 152, no. 5, 1 March 1994 BALTIMORE, MD, USA, pages 2377-2384, C. SIEGALL ET AL. 'In vitro and in vivo characterization of BR96 sFv-PE40.' see abstract --- -/--	1-30

1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 90, no. 16, 15 August 1993 WASHINGTON, DC, USA, pages 7538-7542, U. BRINKMANN ET AL. 'A recombinant immunotoxin containing a disulfide-stabilized Fv fragment.' cited in the application see abstract ---	1-30
A	NATURE, vol. 309, no. 5963, 3 May 1984 LONDON, GB, pages 73-76, H. KLOBECK ET AL. 'Contribution of human VkII germ-line genes to light-chain diversity.' cited in the application see figure 2 ---	1-22, 34-61, 65-88
A	THE JOURNAL OF IMMUNOLOGY, vol. 150, no. 7, 1 April 1993 BALTIMORE, MD, USA, pages 2774-2782, U. BRINKMANN ET AL. 'Recombinant immunotoxins containing the VH or VL domain of monoclonal antibody B3 fused to Pseudomonas exotoxin.' see abstract ---	1-30
A	CANCER RESEARCH, vol. 54, no. 12, 1 July 1994 PHILADELPHIA, PA, USA, pages 3460-3467, M. CHOE ET AL. 'B3(Fab)-PE38M: A recombinant immunotoxin in which a mutant form of Pseudomonas exotoxin is fused to the Fab fragment of monoclonal antibody B3.' see abstract see figure 2 ---	1-30
P,X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 91, no. 25, 6 December 1994 WASHINGTON, DC, USA, pages 12051-12055, I. BENHAR ET AL. 'Rapid humanization of the Fv of monoclonal antibody B3 by using framework exchange of the recombinant immunotoxin B3(Fv)-PE38.' see abstract ---	1-22, 65-88

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	PROTEIN ENGINEERING, vol. 7, no. 12, December 1994 OXFORD, GB, pages 1509-1515, XP 000482989 I. BENHAR ET AL. 'Cloning, expression and characterization of the Fv fragments of the anti-carbohydrate mAbs B1 and B5 as single-chain immunotoxins.' see abstract ---	1-22
P,X	CLINICAL CANCER RESEARCH, vol. 1, no. 9, September 1995 PHILADELPHIA, PA, USA, pages 1023-1029, I. BENHAR ET AL. 'Characterization of B1(Fv)PE38 and B1(dsFv)PE38: Single-chain and disulfide-stabilized Fv immunotoxins with increased activity that cause complete remissions of established hunan carcinoma xenografts in nude mice.' see abstract ---	1-30
P,X	THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 40, 6 October 1995 BALTIMORE, MD, USA, pages 23373-23380, I. BENHAR ET AL. 'Identification of residues that stabilize the single-chain Fv of monoclonal antibodies B3.' see the whole document -----	1-30, 34-64

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/13811

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 30,64,94
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 30,64 and 94 are directed to a method of treatment of the human body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information patent family members

International Application No
PCT/US 95/13811

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9307286	15-04-93	AU-B-	2779892	03-05-93
		CA-A-	2120153	15-04-93
		EP-A-	0610286	17-08-94
		JP-T-	7502643	23-03-95

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